

WHAT IS CLAIMED IS:

1 1. An array of oligonucleotide probes immobilized on a
2 solid support, the array comprising at least two sets of
3 oligonucleotide probes,

4 (1) a first probe set comprising a plurality of
5 probes, each probe comprising a segment of at least three
6 nucleotides exactly complementary to a subsequence of a reference
7 sequence, the segment including at least one interrogation
8 position complementary to a corresponding nucleotide in the
9 reference sequence,

10 (2) a second probe set comprising a corresponding
11 probe for each probe in the first probe set, the corresponding
12 probe in the second probe set being identical to a sequence
13 comprising the corresponding probe from the first probe set or
14 a subsequence of at least three nucleotides thereof that includes
15 the at least one interrogation position, except that the at least
16 one interrogation position is occupied by a different nucleotide
17 in each of the two corresponding probes from the first and second
18 probe sets;

19 wherein the probes in the first probe set have at least
20 three interrogation positions respectively corresponding to each
21 of three contiguous nucleotides in the reference sequence;

22 provided that the array does not contain a complete set
23 of probes of a given length;

24 wherein the reference sequence is from a CFTR gene.

1 2. An array of oligonucleotide probes immobilized on a
2 solid support, the array comprising at least four sets of
3 oligonucleotide probes,

4 (1) a first probe set comprising a plurality of
5 probes, each probe comprising a segment of at least three
6 nucleotides exactly complementary to a subsequence of a reference
7 sequence, the segment including at least one interrogation
8 position complementary to a corresponding nucleotide in the
9 reference sequence,

10 (2) second, third and fourth probe sets, each
11 comprising a corresponding probe for each probe in the first
12 probe set, the probes in the second, third and fourth probe sets
13 being identical to a sequence comprising the corresponding probe

14 from the first probe set or a subsequence of at least three
15 nucleotides thereof that includes the at least one interrogation
16 position, except that the at least one interrogation position is
17 occupied by a different nucleotide in each of the four
18 corresponding probes from the four probe sets;

19 provided the array lacks a complete set of probes of
20 a given length;

21 wherein the reference sequence is from a CFTR gene.

1 3. The oligonucleotide array of claim 2, further
2 comprising a fifth probe set comprising a corresponding probe for
3 each probe in the first probe set, the corresponding probe from
4 the fifth probe set being identical to a sequence comprising the
5 corresponding probe from the first probe set or a subsequence of
6 at least three nucleotides thereof that includes the at least one
7 interrogation position, except that the at least one
8 interrogation position is deleted in the corresponding probe from
9 the fifth probe set.

1 4. The oligonucleotide array of claim 2, further
2 comprising a sixth probe set comprising a corresponding probe for
3 each probe in the first probe set, the corresponding probe from
4 the sixth probe set being identical to a sequence comprising the
5 corresponding probe from the first probe set or a subsequence of
6 at least three nucleotides thereof that includes the at least one
7 interrogation position, except that an additional nucleotide is
8 inserted adjacent to the at least one interrogation position in
9 the corresponding probe from the first probe set.

1 5. The array of claim 2, wherein the first probe set has
2 at least three interrogation positions respectively corresponding
3 to each of three contiguous nucleotides in the reference
4 sequence.

1 6. The array of claim 2, wherein the first probe set has
2 at least 50 interrogation positions respectively corresponding
3 to each of 50 contiguous nucleotides in the reference sequence.

1 7. The oligonucleotide array of claim 2, wherein the array
2 has between 100 and 100,000 probes.

1 8. The oligonucleotide array of claim 2, wherein the
2 probes are linked to the support via a spacer.

1 9. The oligonucleotide array of claim 2, wherein the
2 segment in each probe of the first probe set that is exactly
3 complementary to the subsequence of the reference sequence is 9-
4 21 nucleotides.

1 10. The oligonucleotide array of claim 2, wherein each
2 probe of the first probe set consists of the segment that is
3 exactly complementary to the subsequence of the reference
4 sequence.

1 11. The oligonucleotide array of claim 2, wherein the
2 probes in the second, third and fourth probe sets are identical
3 to the corresponding probe from the first probe set except that
4 the at least one interrogation position is occupied by a
5 different nucleotide in each of the four corresponding probes
6 from the four probe sets.

1 12. An array of oligonucleotide probes immobilized on a
2 solid support, the array comprising at least one pair of first
3 and second probe groups, each group comprising a first and second
4 sets of oligonucleotide probes as defined by claim 1;

5 wherein each probe in the first probe set from the
6 first group is exactly complementary to a subsequence of a first
7 reference sequence and each probe in the first probe set from the
8 second group is exactly complementary to a subsequence from a
9 second reference sequence.

1 13. The array of claim 12, wherein the second reference
2 sequence is a mutated form of the first reference sequence.

1 14. The array of claim 12, wherein each group further
2 comprises third and fourth probe sets, each comprising a

3 corresponding probe for each prob in the first probe set, the
4 probes in the second, third and fourth probe sets being identical
5 to a sequence comprising the corresponding probe from the first
6 probe set or a subsequence of at least three nucleotides thereof
7 that includes the interrogation position, except that the
8 interrogation position is occupied by a different nucleotide in
9 each of the four corresponding probes from the four probe sets.

1 15. The array of claim 14 that comprises at least forty
2 pairs of first and second probe groups, wherein the probes in the
3 first probe sets from the first groups of the forty pairs are
4 exactly complementary to subsequences from forty respective first
5 reference sequences.

1 16. A block of oligonucleotide probes immobilized on a
2 solid support, comprising:

3 a perfectly matched probe comprising a segment of at least
4 three nucleotides exactly complementary to a subsequence of a
5 reference sequence, the segment having a plurality of
6 interrogation positions respectively corresponding to a plurality
7 of nucleotides in the reference sequence,

8 for each interrogation position, three mismatched probes,
9 each identical to a sequence comprising the perfectly matched
10 probe or a subsequence of at least three nucleotides thereof
11 including the plurality of interrogation positions, except in the
12 interrogation position, which is occupied by a different
13 nucleotide in each of the three mismatched probes and the
14 perfectly matched probe;

15 provided the array lacks a complete set of probes of a given
16 length;

17 wherein the reference sequence is from a CFTR gene.

1 17. The array of claim 16, wherein the segment of the
2 perfectly matched probe comprises 3-20 interrogation positions
3 corresponding to 3-20 r spective nucleotides in the reference
4 sequence.

1 18. An array of probes immobilized to a solid support
 2 comprising at least two blocks of probes, each block as defined
 3 by claim 16, a first block comprising a perfectly matched probe
 4 comprising a segment exactly complementary to a subsequence of
 5 a first reference sequence and a second block comprising a
 6 perfectly matched probe comprising a segment exactly
 7 complementary to a subsequence of a second reference sequence.

19. The array of claim 18, wherein the first reference
 sequence is from a wildtype CFTR gene and the second reference
 sequence is from a mutant CFTR gene.

1 20. The array of claim 18, comprising at least 10-100
 2 blocks of probes, each comprising a perfectly matched probe
 3 comprising a segment exactly complementary to a subsequence of
 4 at least 10-100 respective reference sequences:

1 21. An array of oligonucleotide probes immobilized on a
 2 solid support, the array comprising at least four probes:
 3 a first probe comprising first and second segments, each of
 4 at least three nucleotides and exactly complementary to first and
 5 second subsequences of a reference sequence, the segments
 6 including at least one interrogation position corresponding to
 7 a nucleotide in the reference sequence, wherein either (1) the
 8 first and second subsequences are noncontiguous, or (2) the first
 9 and second subsequences are contiguous and the first and second
 10 segments are inverted relative to the complement of the first and
 11 second subsequences in the reference sequence;

12 second, third and fourth probes, identical to a sequence
 13 comprising the first probe or a subsequence thereof comprising
 14 at least three nucleotides from each of the first and second
 15 segments, except in the at least one interrogation position,
 16 which differs in each of the probes;

17 provided the array lacks a complete set of probes of a given
 18 length;

19 wherein the reference sequence is from a CFTR gene.

1 22. A method of comparing a target nucleic acid with a
2 reference sequence comprising a predetermined sequence of
3 nucleotides, the method comprising:

4 (a) hybridizing a sample comprising the target nucleic
5 acid to an array of oligonucleotide probes immobilized on a solid
6 support, the array comprising:

7 (1) a first probe set comprising a plurality of
8 probes, each probe comprising a segment of at least three
9 nucleotides exactly complementary to a subsequence of the
10 reference sequence, the segment including at least one
11 interrogation position complementary to a corresponding
12 nucleotide in the reference sequence, wherein the reference
13 sequence is from a CFTR gene;

14 (2) a second probe set comprising a corresponding
15 probe for each probe in the first probe set, the corresponding
16 probe in the second probe set being identical to a sequence
17 comprising the corresponding probe from the first probe set or
18 a subsequence of at least three nucleotides thereof that includes
19 the at least one interrogation position, except that the at least
20 one interrogation position is occupied by a different nucleotide
21 in each of the two corresponding probes from the first and second
22 probe sets;

23 wherein, the probes in the first probe set have at
24 least three interrogation positions respectively corresponding
25 to each of at least three nucleotides in the reference sequence,
26 and

27 (b) determining which probes, relative to one another, in
28 the first and second probe sets specifically to the target
29 nucleic acid, the relative specific binding of corresponding
30 probes in the first and second probe sets indicating whether a
31 nucleotide in the target sequence is the same or different from
32 the corresponding nucleotide in the reference sequence.

1 23. Th method of claim 22, wherein the determining step
2 comprises:

3 (1) comparing th relative specific binding of two
4 corr sponding probes from the first and second probe s ts;

5 (2) assigning a nucleotide in the target sequence as
6 the complement of the interrogation position of the probe having
7 the greater specific binding;

8 (3) repeating (1) and (2) until each nucleotide of
9 interest in the target sequence has been assigned.

1 24. The method of claim 22, wherein the array further
2 comprises third and fourth probe sets, each comprising a
3 corresponding probe for each probe in the first probe set, the
4 probes in the second, third and fourth probe sets being identical
5 to a sequence comprising the corresponding probe from the first
6 probe set or a subsequence of at least three nucleotides thereof
7 that includes the at least one interrogation position, except
8 that the at least one interrogation position is occupied by a
9 different nucleotide in each of the four corresponding probes
10 from the four probe sets; and the determining step comprises
11 determining which probes, relative to one another, in the first,
12 second, third and fourth probe sets specifically bind to the
13 target nucleic acid, the relative specific binding of
14 corresponding probes in the first, second, third and fourth probe
15 sets indicating whether a nucleotide in the target sequence is
16 the same or different from the corresponding nucleotide in the
17 reference sequence.

1 25. The method of claim 24, wherein the determining
2 comprises:

3 (1) comparing the relative specific binding of four
4 corresponding probes from the first, second, third and fourth
5 probe sets;

6 (2) assigning a nucleotide in the target sequence as
7 the complement of the interrogation position of the probe having
8 the greatest specific binding;

9 (3) repeating (1) and (2) until each nucleotide of
10 interest in the target sequence has been assigned.

1 26. A method of comparing a target nucleic acid with a
2 reference sequence comprising a predetermined sequence of
3 nucleotides, the method comprising:

4 (a) hybridizing the target nucleic acid to an array
5 of oligonucleotide probes immobilized on a solid support, the
6 array comprising:

7 a perfectly matched probe comprising a segment of at least
8 three nucleotides exactly complementary to a subsequence of a
9 reference sequence, the segment having a plurality of
10 interrogation positions respectively corresponding to a plurality
11 of nucleotides in the reference sequence, wherein the reference
12 sequence is from a CFTR gene;

13 for each interrogation position, three mismatched probes,
14 each identical to a sequence comprising the perfectly matched
15 probe or a subsequence of at least three nucleotides thereof
16 including the plurality of interrogation positions, except in the
17 interrogation position, which is occupied by a different
18 nucleotide in each of the three mismatched probes and the
19 perfectly matched probe;

20 (b) for each interrogation position,

21 (1) comparing the relative specific binding of the
22 three mismatched probes and the perfectly matched probe;

23 (2) assigning a nucleotide in the target sequence as
24 the complement of the interrogation position of the probe having
25 the greatest specific binding.

1 27. The method of claim 26, wherein the target sequence has
2 an undetermined substitution relative to the reference sequence,
3 and the method assigns a nucleotide to the substitution.

1 28. A method of comparing a target nucleic acid with a
2 reference sequence comprising a predetermined sequence of
3 nucleotides, the method comprising:

4 hybridizing the target sequence to the array of claim
5 19;

6 determining which probes in the first group, relative
7 to one another, hybridize to the target sequence, the relative
8 specific binding of the probes indicating whether the target
9 sequence is the same or different from the first reference
10 sequence;

11 d terminating which probes in th second group, relative
12 to one another, hybridize to the target sequence, the relative
13 specific binding of the probes indicating whether the target
14 sequence is the same or different from the second reference
15 sequence.

1 29. The method of claim 27, wherein the hybridizing step
2 comprising hybridizing the target sequence and a second target
3 sequence to the array, and the relative specific binding of the
4 probes from the first group indicates that the target is
5 identical to the first reference sequence, and the relative
6 specific binding of the probes from the second group indicates
7 that the second target sequence is identical to the second
8 reference sequence.

1 30. The method of claim 29, wherein the first and second
2 target sequences are heterozygous alleles.

add A2

add
A1